The Effect of Exogenous Energy Sources on the Synthesis of β-Galactosidase in Resting-Cell Suspensions of *Escherichia coli*

I. S. PALMER and M. F. MALLETTE

From the Department of Agricultural and Biological Chemistry, The Pennsylvania State University, University Park. Dr. Palmer's present address is 1602 South West Street, Falls Church, Virginia

ABSTRACT Using methyl-1-thio-β-D-galactoside as the inducer, the biosynthesis of β-galactosidase was observed in *Escherichia coli* B with only endogenous sources of nitrogen and energy available. The addition of glucose, ribose, xylose, or glycerol as exogenous energy sources to nitrogen-deficient media blocked enzyme formation. Preinduction of the resting cells failed to overcome inhibition by the added energy sources. With limited quantities of glucose, ribose, xylose, or glycerol, synthesis of β-galactosidase resumed abruptly and continued at the rate normal for cells in nitrogen-deficient media. Comparison of enzyme activities with oxygen uptake data revealed a reduction in the rate of oxygen uptake at the time enzyme synthesis resumed in media originally containing small amounts of energy sources. This change corresponded to only a fraction of the oxygen required for complete oxidation of one of the exogenous substrates. It is suggested that inhibition by these particular exogenous substrates involves metabolism to a common repressor or interference with an energy-transfer system.

The inhibition of induced enzyme synthesis by added glucose was reported several years ago by Monod (1) and Gale (2). Rickenberg and Lester (3) studied the preferential synthesis of β-galactosidase in *Escherichia coli* and suggested that unknown endogenous materials serve as energy sources instead of exogenous materials. At the same time, Pardee (4) observed that glycerol blocks the formation of β-galactosidase in pyrimidineless mutants in media free of pyrimidine. He concluded that glycerol blocked the biosynthesis by causing exhaustion of the internal pool of pyrimidines. Mandelstam (5) demonstrated β-galactosidase synthesis by *E. coli* in N-deficient media

and discovered that the turnover of protein was great enough to account for the enzyme obtained.

Neidhard and Magasanik (6) reported that energy sources other than glucose also inhibit enzyme synthesis in growing cultures but that the maximum inhibition observed was 75 per cent of that for glucose. Recently Cohn and Horibata (7) likewise reported inhibition by several energy sources. Only glucose and mannitol were completely inhibitory. Magasanik (8) has reviewed the interactions of nitrogen and energy sources with induced enzyme synthesis.

Of the previous work only that of Pardee (4) specifically involved the inhibition of enzyme synthesis by an exogenous energy source added to resting cells. In the present study the inhibitions by a series of exogenous energy sources were compared in nitrogen-deficient media. It was found that xylose, ribose, glycerol, and glucose all completely inhibited the formation of β-galactosidase in such a way as to warrant reconsideration of the inhibitory mechanism.

MATERIALS AND METHODS

Growth and Experimental Media *Escherichia coli* strain B was used throughout this work. The cells were grown at pH 7.1 on a synthetic medium with the following composition: 0.6 per cent Na_2HPO_4, 0.3 per cent KH_2PO_4, 0.1 per cent NH_4Cl, 0.05 per cent NaCl, 0.041 per cent $MgSO_4 \cdot 7H_2O$ and an energy source added to give a final concentration of 0.4 per cent. Cells were grown at 37°C to a population of 4 to 5 × 10^8 cells per ml in 22 × 175 mm culture tubes containing 10 ml of the synthetic medium aerated by shaking at 200 cycles per minute and at an amplitude of 2 cm. Culture turbidity, measured on a Coleman model 14 universal spectrophotometer at 650 mμ and calibrated against plate and microscopic cell counts, was used to follow growth.

Nitrogen-deficient media for maintenance of non-growing populations were prepared by omitting the NH_4Cl from the above solution of salts and by adding any desired energy source. Studies in such media were conducted by incubation in 16 × 125 mm culture tubes each containing 5 ml of medium.

Washing Since much of this work employed resting cells, complete washing was very important. The cultures were centrifuged at 1200 × *g* and 4°C for 15 minutes, and the pellet of cells was suspended in 10 ml of a cold solution containing all the salts of the synthetic medium except NH_4Cl. These suspensions were then combined in one tube. After recentrifuging, the pellet of cells was washed three more times by suspending it in 10 ml of the same solution of salts and centrifuging as above. After the final wash, the cells were resuspended in the experimental medium and divided among several culture tubes so that the final concentration in 5 ml of N-deficient medium was 4 to 5 × 10^8 cells per ml. The turbidity was then measured to determine the actual number of cells in the experiment.

Enzyme Induction and Assay Induction of β-galactosidase was carried out in a deficient medium containing the nitrogen-free solution of salts and an appropriate inducer. Two inducers were utilized. Merck U.S.P. lactose was added at a concentration of 0.8 per cent. Methyl-1-thio-β-D-galactoside (methylthiogalactoside) was used in other experiments at a concentration of 10^{-3} M. The latter compound was prepared by the procedure of Helferich and Türk (9) except that the intermediate, α-bromotetraacetylgalactose, was prepared by the method of Barczai-Martos and Kőrösy (10).

Lysates of cells were used in all the enzyme assays. Lysis was accomplished by incubating the cells for 20 minutes at 37°C with T_{2r+} phage[1] a at concentration of 100 phage particles per bacterial cell.

The β-galactosidase in induced cells was assayed by a modification of the Lederberg (11) method. Cell lysate (0.10 or 0.20 ml depending on enzyme concentration) was added to enough 0.05 M sodium phosphate buffer (pH 7.1) to give a final volume of 6.0 ml. The reaction tube was incubated for 10 minutes at 37°C and then *o*-nitrophenyl-β-D-galactoside was added to give a concentration of 1.25×10^{-3} M in 8.0 ml of reaction mixture. After shaking for 20 minutes at 37°C, 2.0 ml. of 1 M K_2CO_3 was added. The hydrolysis mixture was read against a reagent blank at 420 mμ on a Coleman 14 universal spectrophotometer. A standard curve was prepared by hydrolyzing known amounts of *o*-nitrophenyl-β-D-galactoside with an excess of enzyme and plotting absorbancy *versus* *o*-nitrophenyl-β-D-galactoside concentration. A unit of enzyme activity was defined as that amount of enzyme producing the hydrolysis of 0.1 mole *o*-nitrophenyl-β-D-galactoside per minute per milligram of dry cell weight.

Dry Weight Cells were grown on glucose-synthetic medium (a total of 3.6×10^{10} cells) and were washed twice with water. The washed cells were placed in a small weighing vessel and dried to constant weight *in vacuo* at 80°C. One mg of dry material contained 3.24×10^9 cells.

Simultaneous Studies of Enzyme Synthesis and Substrate Oxidation Cells were grown in the synthetic medium containing the desired energy source, washed, and then suspended in 5 ml of N-deficient medium containing methylthiogalactoside and a designated amount of a particular energy source. A series of such systems was incubated at 37°C and at intervals tubes were removed, placed in an ice bath, and then assayed for enzyme activity. Other aliquots of cells from the same cultures were placed in the sidearms of Warburg flasks. The flasks contained N-deficient medium and an energy source equal in amount to that in the culture tubes used in following enzyme synthesis. After a 10 minute equilibration period, the cells were tipped into the solution of the energy source and oxygen uptake was determined. The zero time for the cells used in the Warburg studies was 20 to 30 minutes after that for the cells used in the enzyme assay. With this exception, the cells in both systems were identical.

[1] The bacteriophage was kindly supplied in concentrated suspension by Dr. James L. Barlow of the New York State Department of Health, Albany, New York.

EXPERIMENTAL RESULTS

In recent years, it has been demonstrated by workers in different laboratories (5, 12, 13) that organisms with small metabolic reserves, such as *E. coli*, can synthesize enzyme in a nitrogen-free medium. A study of the energy requirements of such a system might be of interest. Therefore, cells were grown on glucose-synthetic medium, washed, and divided into three lots. One group of cells was exposed to a nitrogen-deficient (N-deficient) medium containing 10^{-3} M methylthiogalactoside but no energy source. The second batch was exposed to N-deficient medium containing 0.8 per cent lactose serving as both an inducer and an energy source. The third batch was exposed to N-deficient medium containing both inducers.

TABLE I

β-GALACTOSIDASE SYNTHESIS IN RESTING MEDIA BY CELLS GROWN ON GLUCOSE-SYNTHETIC MEDIUM

Cells grown on glucose-synthetic medium were washed and suspended at a concentration of 4.4×10^8 cells per ml in resting media containing the indicated inducers. After a 3 hour exposure period, the cells were lysed and assayed for β-galactosidase activity.

	Inducer		
	10^{-4} M MTG*	0.8 per cent lactose	0.8 per cent lactose 10^{-3} M MTG*
Units of enzyme activity	8.26‡	1.92	2.64
	8.55	2.05	2.46

* Methylthiogalactoside.

‡ The pair of results in each column represents means of duplicates in duplicate experiments.

The data in Table I show that more enzyme synthesis took place in the energy-free medium than in either of the other two media containing lactose. Since methylthiogalactoside is known (14) to be a stronger inducer than lactose, the difference in the amounts of enzyme induced by the two different compounds might have been due to a difference in inducer efficiency. The data in the third column of Table I indicate that this is not the explanation. The amount of enzyme formed in the system of combined inducers should have been similar to the amount produced in the system with only methylthiogalactoside if only a difference in inducer efficiency were involved. Instead, the data indicate that lactose actually inhibits enzyme formation.

It is evident from the data presented that this strain of *E. coli* cells can synthesize enzyme in the absence of both exogenous nitrogen and energy as observed by Mandelstam (5) with another strain. This capacity for enzyme synthesis must be dependent upon readily available metabolic pools since

the capacity was changed markedly by washing or starvation. Six additional washings or a 2 hour starvation period were each found adequate to reduce the enzyme-synthesizing capacity of the cells to 40 per cent of the original value. Starvation was carried out by exposing the cells at 37°C to a medium containing nitrogen but lacking an energy source.

Since the capacity of cells for enzyme synthesis in a deficient medium appeared dependent upon the metabolic pools, it seemed probable that the original energy source used for growth determined the size of the pools and thereby affected the capacity for enzyme synthesis. To check this point, cells were grown on synthetic media containing various sources of energy and then induced in a N-deficient medium containing methylthiogalactoside but no energy source. As shown in Table II, there is some correlation between

TABLE II

INDUCTION OF β-GALACTOSIDASE IN RESTING MEDIA BY 10^{-3} M METHYLTHIOGALACTOSIDE IN CELLS GROWN ON VARIOUS ENERGY SOURCES

Cells were grown to populations of 4.8×10^8 cells per ml in synthetic media containing the designated energy sources. After washing, the cells were then exposed to resting medium containing 10^{-3} M methylthiogalactoside but no energy source. After 3 hours, the cells were lysed and assayed for β-galactosidase activity.

	Glucose	Xylose	Acetate	Alanine	Glycerol
Generation time, *min.*	55	66	202	122	67
Units of enzyme activity	5.00	4.41	3.46	3.53	6.05
	6.05	4.79	3.19	3.90	6.05

the rate at which substances supported growth and the capacity for enzyme synthesis by cells grown on these energy sources. In general, cells grown on substances which supported growth at the fastest rates, formed the most enzyme in resting medium. However, differences in enzyme-forming capacities were not as large as might be expected. It therefore appeared that the original source of energy may not be critical in establishing the sizes of the metabolic pools.

The possibility still existed, however, that the presence of some of these energy sources during induction of enzyme synthesis in the deficient medium might stimulate enzyme production by maintaining high metabolite levels in the storage pools. On the other hand, energy sources might cause inhibition similar to that obtained with lactose. Hence, cells were grown on glucose-synthetic medium and then transferred to N-deficient media containing 10^{-3} M methylthiogalactoside and the various energy sources designated in Table III. The results shown in the upper part of Table III are consistent

with the data in Table I because the largest enzyme production occurred in cells suspended in the energy-free medium. Since enzyme activity in uninduced cells was found to be approximately 0.10 unit, it is evident that the presence of glucose in the induction medium caused complete inhibition of enzyme synthesis. The presence of other energy sources caused various degrees of inhibition.

From previous experience, it was known that *E. coli* must be adapted to several of the compounds considered in Table III before they can be utilized for growth. Hence the inhibition observed in some instances might not have been maximal since the inhibitory substances actually were not available

TABLE III

β-GALACTOSIDASE ACTIVITY FROM RESTING CELLS INDUCED WITH 10^{-3} M METHYLTHIOGALACTOSIDE IN THE PRESENCE OF VARIOUS ENERGY SOURCES

Cells grown on	Exogenous energy source during induction						
	None	Glucose	Xylose	Pyruvate	Glycerol	Acetate	Lactose
Glucose	5.23	0.11	2.67	—	1.96	4.48	2.60
	5.90	0.07	2.71	—	1.79	4.66	3.05
Xylose			0.15				
			0.19				
Pyruvate				0.49			
				0.45			
Glycerol					0.28		
					0.32		
Acetate						1.10	
						1.40	
Lactose							0.88
							0.79

energy sources for the early parts of the 3 hour experiments with cells grown on glucose and tested on other energy sources. To evaluate this possibility, cells were grown on the energy source to which they were later exposed in the N-deficient medium. Under these conditions, inhibition caused by the presence of either glycerol or xylose was almost equivalent to that caused by glucose (lower part of Table III). In repeated experiments the inhibition equaled that by glucose. Hence complete inhibition of enzyme synthesis is not an exclusive property of glucose but is exhibited by substrates as different as xylose and glycerol, at least under the conditions employed in this work.

Cohn and Monod (15) and Cohn and Horibata (7) demonstrated that preinduction of growing cells for β-galactosidase synthesis causes them to become partially resistant to the inhibitory effects of glucose. Therefore, it

seemed of value to measure the effect of preinduction on inhibition in resting cells.

Cells were grown on glucose-synthetic medium, washed, and placed in N-deficient medium containing 10^{-3} M methylthiogalactoside. The cells were divided into four groups, one of which served as a control for enzyme synthesis. Glucose was added to the other three groups (final concentration of 0.4 per cent) at the times indicated by the arrows in Fig. 1. It can be seen that the simultaneous addition of the glucose and inducer at zero time resulted in complete inhibition as before. The addition of glucose to cells

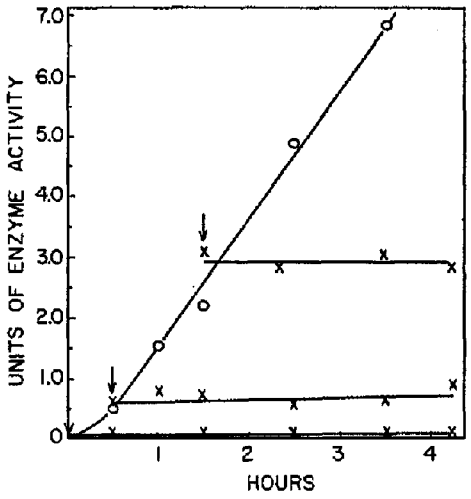

FIGURE 1. Inhibition by the addition of 0.4 per cent glucose to cells in which β-galactosidase synthesis was preinduced. Cells were grown on glucose-synthetic medium, washed, and suspended in nitrogen-free resting medium containing 10^{-3} M methylthiogalactoside. O, no glucose added; X, glucose added at the times indicated by arrows.

previously exposed to inducer for 30 minutes still resulted in abrupt and complete inhibition. The results were the same for cells which had been preinduced for 90 minutes and differ from those cited above for growing cells.

When cells grown on xylose-synthetic medium were placed in resting medium containing methylthiogalactoside, it was found that addition of xylose to preinduced cells again caused instantaneous and complete inhibition of enzyme synthesis. In an identical type of experiment, the results obtained with glycerol or ribose were similar with the exception that a lag of approximately 10 minutes was required before inhibition of enzyme synthesis became complete. A typical experiment with ribose is shown in Fig. 2.

Since the inhibition by the various energy sources was so severe and immediate, the question of persistence of the inhibition arose. When limited concentrations of glucose were used, however, inhibition ceased after a period

of incubation as shown in Fig. 3. All cells were grown on glucose-synthetic medium, washed, and divided into four groups. Each group was exposed to a N-deficient medium containing 10^{-3} M methylthiogalactoside and the amount of glucose designated in Fig. 3. A high concentration of glucose (0.4 per cent) caused complete inhibition for the duration of the experiment. Limited concentrations caused complete inhibition of synthesis for a short time, but enzyme production recommenced at the same rate as in the control group (0.0 per cent glucose). Similar families of curves were obtained with glycerol, xylose, and ribose as the energy sources in place of glucose. Higher concentrations of these other substances were required to bring about the duration of inhibition observed with glucose.

Recovery from the inhibition of enzyme synthesis in media originally containing low concentrations of energy sources made it appear as if the energy

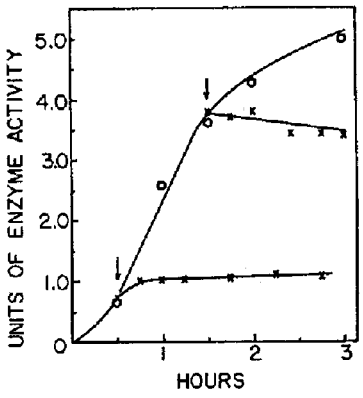

FIGURE 2. Inhibition by the addition of 0.4 per cent ribose to cells in which α-galactosidase synthesis was preinduced. Cells were grown on ribose-synthetic medium, washed, and suspended in nitrogen-free resting medium containing 10^{-3} M methylthiogalactoside. ○, no ribose was added; ×, ribose was added at the times indicated by arrows.

source was merely depleted by the normal cellular metabolism. Thereafter, normal enzyme synthesis resumed. This idea led to the simultaneous study of enzyme synthesis and oxygen uptake in order to determine the extent of the metabolism of the various substrates at the point where inhibition ends.

Fig. 4 shows data obtained from such an experiment involving the use of limited glucose concentrations. Enzyme synthesis was only temporarily inhibited as demonstrated previously by the data in Fig. 3. The oxygen uptakes were relatively rapid at the start. At times corresponding to the terminations of the inhibition of enzyme synthesis, the rate of oxygen uptake decreased to a new rate. The decrease in rate occurred sharply, but the new rate was still higher than that for endogenous respiration. The state of substrate oxidation at the point where inhibition of enzyme synthesis ceased was estimated by extrapolation to the ordinate (assuming the usual endogenous uptake of oxygen). About 1.5 moles of oxygen per mole of glucose was consumed during the rapid metabolic process and was independent of the glucose

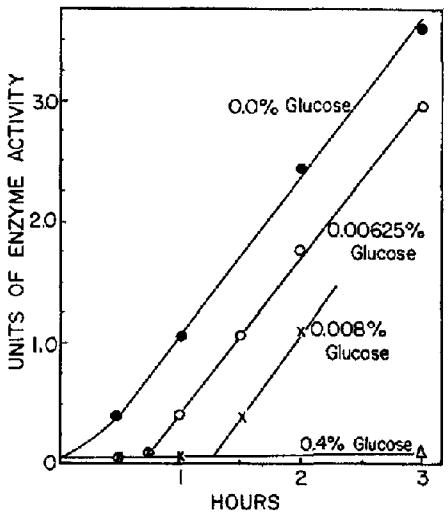

FIGURE 3. Temporary inhibition of enzyme synthesis by low concentrations of glucose. Cells were grown on glucose-synthetic medium, washed, and divided into four groups which were each suspended in nitrogen-free resting medium containing 10^{-3} M methylthiogalactoside. In addition, the media used for the three experimental groups contained the amounts of glucose shown.

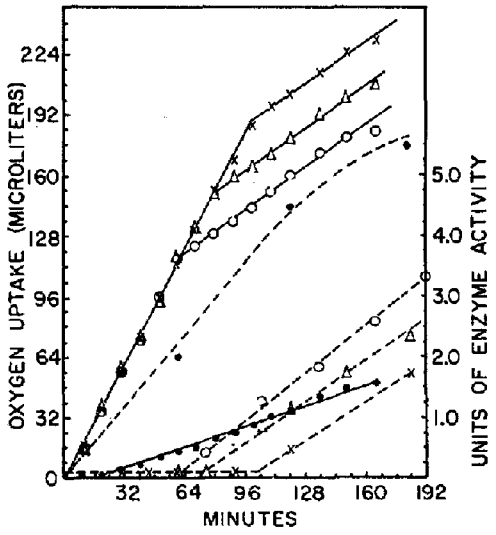

FIGURE 4. Oxygen uptake and enzyme synthesis in the presence of various amounts of glucose. Cells were grown on glucose-synthetic medium and washed. Equal numbers of cells were suspended in culture tubes and Warburg flasks, each of which contained nitrogen-free medium with methylthiogalactoside and the designated amount of glucose. ●, no glucose; ○, 1.74 micromoles of glucose; △, 2.22 micromoles of glucose; ×, 2.78 micromoles of glucose; ----, enzyme synthesis; ——, oxygen uptake.

concentration used. This low uptake indicates that the glucose is far from completely oxidized when inhibition ceases.

Similar expriments were run with glycerol and ribose with results much like those obtained with glucose save for a few exceptions in detail. Different

TABLE IV

EXTENT OF SUBSTRATE OXIDATION AT CESSATION OF INHIBITION OF ENZYME SYNTHESIS

The ratios shown below were obtained by extrapolation of curves such as those of Figs. 4 and 5. The portion of the oxidation curve beginning at the cessation of enzyme inhibition was extrapolated to the ordinate.

Substrate	Moles O_2 per mole of substrate*
Glucose	1.50–1.72
Xylose	1.36–1.60
Glycerol	0.99–1.11
Ribose	1.06–1.22

* Values represent the ranges obtained from several experiments.

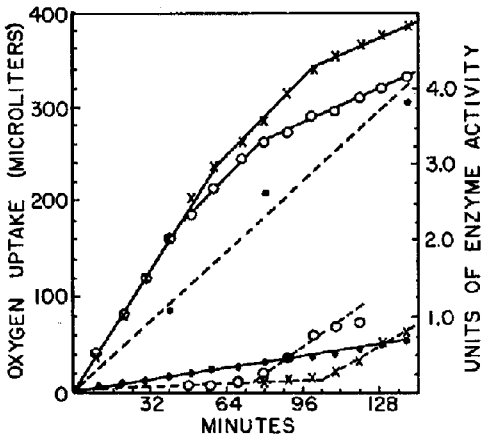

FIGURE 5. Oxygen uptake and enzyme synthesis in the presence of xylose. Cells were grown on xylose-synthetic medium and washed. Equal numbers of cells were suspended in culture tubes and Warburg flasks each of which contained nitrogen-free medium with methylthiogalactoside and the designated amount of xylose. ●, no xylose; ○, 5.00 micromoles of xylose; ×, 6.67 micromoles of xylose; -----, enzyme synthesis; ——, oxygen uptake.

concentrations of the other energy sources were required to produce the duration of inhibition obtained with glucose. Furthermore, different ratios of oxygen consumed per mole of energy source were observed at cessation of inhibition. These ratios are shown in Table IV.

When an experiment of the above type was conducted with xylose as

shown in Fig. 5, cells utilizing xylose displayed three different phases in the oxygen uptake curves. In this instance the cessation of inhibition coincided with the commencement of the third phase in the oxygen uptake curve instead of the second as with the other energy sources. However, the general pattern of the inhibition of enzyme synthesis by the energy sources appeared similar regardless of the substance used. A decrease in the rate of oxygen consumption always accompanied the termination of inhibition of enzyme synthesis.

DISCUSSION

The foregoing data show that the endogenous sources of energy and nitrogen are adequate for β-galactosidase synthesis in resting cells. Furthermore, the addition of an exogenous energy source appears to cause some depression in the amount of enzyme synthesized. When the cells were grown on an energy source prior to exposure to it in N-deficient medium containing inducer, inhibition by some substrates was severe. Xylose, ribose, and glycerol completely inhibited enzyme synthesis. Hence complete inhibition is not an exclusive property of glucose as has been implied by some workers (6).

Cohn and Monod (15) and recently Cohn and Horibata (7) have reported that the exposure of growing *E. coli* cells to inducer prior to the addition of an inhibitory substance such as glucose caused their strains of *E. coli* to become partially resistant to inhibition by glucose.

It is apparent that the present resting cells behave differently than did the growing cells of Cohn. Glucose stopped enzyme synthesis immediately although induction was allowed to proceed for as long as 90 minutes before the addition of the glucose. Since the formation of the entry system should have been complete, the mechanism of inhibition in resting cells is apparently not simply an interference with the biosynthesis of a permease. Otherwise the preinduced resting cells should have been resistant to glucose inhibition. Furthermore, a direct interaction between the inhibitory carbohydrates and the β-galactoside permease may not account for the inhibition observed since it seems unlikely that four different energy sources should all react in the same way with the permease.

Inhibition by added substrate was always greatest in cells previously grown on that substrate (Table III). In such adapted cells the enzyme systems involved in the utilization of the substrates should have been at maximum levels and little further synthesis of these enzymes would be expected or needed in the resting systems. In other words, there should be little competition for the amino acids needed in the biosynthesis of β-galactosidase.

One might expect recovery from inhibition of enzyme synthesis only when a limited concentration of the exogenous energy source is completely metabo-

lized and the cells have returned to their normal endogenous metabolism. However, the states of substrate oxidation at the times corresponding to the end of inhibition (Table IV) show that energy sources were only partially oxidized. Thus some of the intermediates in the pools may be at high enough concentrations for inhibition of enzyme induction. In addition, the coincidence of enzyme synthesis with a change in oxygen uptake suggests that the four substrates might be passing along metabolic routes with steps in common which repress enzyme formation as suggested by Cohn and Horibata (7) and Pardee *et al.* (16). The decrease in oxygen uptake shown in Figs. 4 and 5 could then be taken to represent metabolic consumption of this inhibitory substance(s).

It appears that at least one such postulated inhibitor (repressor) might follow glycerol in the normal catabolic schemes, since glycerol, as well as the three carbohydrates above it, caused complete inhibition. Magasanik and Bojarska (17) postulated that gluconate or subsequent compounds are the repressor substances inhibiting enzyme synthesis in *Aerobacter aerogenes*. An attempt (Table IV) to elucidate the nature of the inhibitory substance by a comparison of the states of oxidation of the various substrates at the point where enzyme inhibition ceased was unsuccessful because of inadequate knowledge of the network of metabolic pathways involved. Radioactive substrates might facilitate the detection of an intermediate repressor material.

The data presented here do not exclude the possibility that the inhibition may result from interference with or by an energy-transferring substance. Thus the sudden addition of substrate may temporarily reduce the concentration of an energy-transferring intermediate directly or indirectly necessary for enzyme synthesis. Indeed, the data suggest that only endogenous energy sources are effective in the biosynthesis of β-galactosidase and that the repressor(s) somehow prevents this utilization, perhaps by temporary depletion of a key intermediate.

It should be noted that different states of oxidation of ribose and xylose are required to obtain reversal of enzyme inhibition. This difference is unexpected since these two substances should be readily interconverted and hence metabolized by a common pathway. Moreover, the rate of oxygen uptake for cells utilizing xylose passed through three phases while cells utilizing ribose exhibited only two oxidative rates at the concentrations employed here. These two factors, in conjunction with the observation that it was much more difficult to adapt cells to growth on ribose than on xylose, lead to the speculation that there may be separate pathways for the uptake or the utilization of ribose and xylose in resting cells of *E. coli*.

This paper was authorized for publication on October 18, 1960, as paper No. 2500 in the journal series of the Pennsylvania Agricultural Experiment Station.
Received for publication, March 20, 1961.

REFERENCES

1. MONOD, J., Thesis at Sorbonne University, Paris, Hermann et Cie., 1941.
2. GALE, E. F., *Bact. Rev.*, 1943, **7**, 139.
3. RICKENBERG, H. V., and LESTER, G., *J. Gen. Microbiol.*, 1955, **13**, 279.
4. PARDEE, A. B., *J. Bact.*, 1955, **69**, 233.
5. MANDELSTAM, J., *Nature*, 1957, **179**, 1179.
6. NEIDHARDT, F. C., AND MAGASANIK, B., *J. Bact.*, 1957, **73**, 260.
7. COHN, M., and HORIBATA, K., *J. Bact.*, 1959, **78**, 624.
8. MAGASANIK, B., *Ann. Rev. Microbiol.*, 1957, **11**, 221.
9. HELFERICH, B., and TÜRK, D., *Ber. chem. Ges.*, 1956, **89**, 2215.
10. BARCZAI-MARTOS, H., and KŐRÖSY, F., *Nature*, 1950, **165**, 369.
11. LEDERBERG, J., *J. Bact.*, 1950, **60**, 381.
12. LØVTRUP, S., *Biochim. et Biophysica Acta*, 1956, **19**, 247.
13. WEINBAUM, G., and MALLETTE, M. F., *J. Gen. Physiol.*, 1959, **42**, 1207.
14. MONOD, J., *in* Enzymes: Units of Biological Structure and Function, (H. O. Gaebler, editor), New York, Academic Press, Inc., 1956, 7.
15. COHN, M., and MONOD, J., *Third Symp. Soc. Gen. Microbiol.*, 1953, 132.
16. PARDEE, A. B., JACOB, F., AND MONOD, J., *Compt. rend. Acad. sc.*, 1958, **246**, 3125.
17. MAGASANIK, A. K., and BOJARSKA, A., *Biochem. and Biophysical Research Comm.*, 1960, **2**, 77.